



**UNIVERSITI PUTRA MALAYSIA**

**THE EFFICACY OF THREE SPECIES OF TRICHODEW FOR  
THE CONTROL OF BASAL STEM ROT IN OIL PALM SEEDLINGS**

**JAYANTHI NAGAPPAN**

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**THE EFFICACY OF THREE SPECIES OF *TRICHODERMA* FOR THE  
CONTROL OF BASAL STEM ROT IN OIL PALM SEEDLINGS**

**By**

**JAYANTHI NAGAPPAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in  
Fulfilment of the Requirements for the Degree of Masters of Science**

**February 2005**



*To my beloved parents for their love and patience and my twin sister,  
Jayasree for her moral support throughout my studies*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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**February 2005**

**Chairman: Associate Professor Faridah Abdullah, PhD**

**Faculty: Science**

This study evaluated the potential of three *Trichoderma* species, namely *T. harzianum* (isolate BIO T32), *T. longibrachiatum* (BIO T28) and *T. virens* (BIO T128) for the control of *Ganoderma boninense* (EGB 01), the causal pathogen of basal stem rot (BSR) of oil palms in nursery trials. Besides their spore production and antagonistic properties, this study also investigated the growth response of each of the species towards a wide range of temperature and pH conditions. All three species exhibited particular strengths in the growth parameters studied but BIO T32 exhibited consistent and relatively good antagonistic properties and was used as the main inoculant in nursery trials against *G. boninense*. The type and size of wood block were found to influence the success and consistency of the inocula in establishing disease during artificial infection of seedlings. Very low infectivity rates were achieved when inoculum blocks were half to a quarter of the standard 6 x 6 x 12 cm; this size was found to give consistent infection rates leading to approximately 85% mortality. In

nursery trials, seedlings treated with a single inoculum of *T. harzianum* (T1) gave the lowest and most significant disease severity index (DSI) of 28.34. The conidial drench was stopped at week 14 and the first sign of disease was only observed on week 20. The uninfected and untreated control seedlings gave a DSI of 0 where as, the infected, untreated controls gave a DSI of 86.87. Soils under treatment using a single (T1), two mixed (T2) and three mixed (T3) inocula showed an increase in spore count based on colony forming units (cfu) starting from two weeks after application. When the soil drench was terminated at week 14, the spore count was peak on the 18<sup>th</sup>, 14<sup>th</sup> and 10<sup>th</sup> week for T1, T2 and T3 treatments respectively. Spore counts of BIO T32 were not significantly different on the upper (5 cm) and deeper (15 cm) layer of the treated soils. This study found that when *T. harzianum* (BIO T32) was used as a single inoculum, it gave the most significant and effective performance as a biological control agent. This was only followed by a mixture of *T. harzianum* and *T. longibrachiatum*. Lastly, the use of a combination of three *Trichoderma* species were found to give the poorest disease control, giving a DSI that was not statistically different from the infected, untreated control experiment.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains

**KEUPAYAAN TIGA SPESIS *TRICHODERMA* BAGI PENGAWALAN  
PENYAKIT REPUT PANGKAL BATANG ANAK POKOK KELAPA SAWIT**

Oleh

**JAYANTHI NAGAPPAN**

**Februari 2005**

**Pengerusi: Profesor Madya Faridah Abdullah, PhD**

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Kajian ini menilai potensi tiga spesis *Trichoderma*, terutamanya *T. harzianum* (isolat BIO T32), *T. longibrachiatum* (BIO T28) dan *T. virens* (BIO T128) sebagai kawalan terhadap *Ganoderma boninense* (EGB 01), patogen reput pangkal batang (BSR) pokok kelapa sawit dalam kajian nurseri. Selain penghasilan spora dan ciri-ciri relatif antagonis mereka, kajian ini juga menilai tindakbalas ketiga-tiga spesis ini terhadap julat suhu dan pH yang luas. Ketiga-tiga spesis tersebut mempamerkan ciri-ciri tertentu dalam parameter yang dikaji tetapi BIO T32 mempamerkan ciri relatif antagonis yang baik dan konsisten serta dipilih sebagai inokulan utama dalam kajian nurseri terhadap *G. boninense*. Jenis dan saiz blok kayu didapati mempengaruhi kejayaan dan konsistensi inokula dalam memperkukuhkan penyakit semasa jangkitan secara buatan terhadap anak pokok kelapa sawit. Kadar jangkitan yang amat rendah diperolehi dengan blok inokulum yang bersaiz kecil berbanding dengan saiz 6 x 6 x 12 cm; saiz ini didapati memberi kadar jangkitan yang konsisten sehingga 85% kematian. Dalam kajian

nurseri, anak pokok yang dirawat dengan sejenis aplikasi inokulum *T. harzianum* (BIO T32) memberikan tahap kemerosotan penyakit (TKP) yang teramat rendah dan signifikasi iaitu sebanyak 28.34. Penggunaan cecair konidia ditamatkan pada minggu ke-14 dan kesan jangkitan hanya diperhatikan pada minggu ke-20. Anak pokok kawalan yang tidak dijangkiti dan tidak dirawat memberi nilai TKP 0, tetapi kawalan yang dijangkiti dan tidak dirawat memberi nilai TKP sebanyak 86.87. Tanah yang dirawat dengan satu (T1), kombinasi dua (T2) dan kombinasi tiga (T3) jenis inokula menunjukkan kenaikan kiraan spora berdasarkan unit pembentuk koloni (upk) yang bermula dari minggu ke-2 selepas aplikasinya. Apabila aplikasi larutan inokulum ditamatkan pada minggu ke-14, kiraan spora memuncak pada minggu ke 18, 14 dan 10 untuk rawatan T1, T2 dan T3 masing-masing. Kiraan spora BIO T32 tidak signifikan pada tahap atas (5 cm) dan dalam (15 cm) tanah yang dirawat. Kajian ini mendapati apabila sejenis inokulum *T. harzianum* (BIO T32) digunakan, ia sangat signifikan dan efektif sebagai agen kawalan biologi. Ini diikuti dengan kombinasi *T. harzianum* dan *T. longibrachiatum*. Akhirnya, kombinasi ketiga-tiga spesis *Trichoderma* didapati memberikan pengawalan penyakit yang tidak memuaskan dengan TKP yang tidak signifikan dari anak pokok kawalan yang dijangkiti dan tidak dirawat.

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I certify that an Examination Committee met on 3<sup>rd</sup> February 2005 to conduct the final examination of Jayanthi Nagappan on her Master of Science thesis entitled “The Efficacy of Three Species of *Trichoderma* for the Control of Basal Stem Rot in Oil Palm Seedlings” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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**DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

  
**JAYANTHI NAGAPPAN**

Date: 18 MAY 2005

## TABLE OF CONTENTS

	Page
DEDICATION	2
ABSTRACT	3
ABSTRAK	5
ACKNOWLEDGEMENTS	7
APPROVAL	8
DECLARATION	10
LIST OF TABLES	14
LIST OF FIGURES	16
LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS	19
 <b>CHAPTER</b>	
 <b>I. INTRODUCTION</b>	 21
 <b>II. LITERATURE REVIEW</b>	
The oil palm	28
Occurrence of basal stem rot of oil palm	29
Symptoms of basal stem rot of oil palm	30
Taxonomic studies of <i>Ganoderma</i>	31
Pathogenicity studies of <i>Ganoderma boninense</i>	33
Control of basal stem rot	33
Biological control	37
Taxonomy and morphology of <i>Trichoderma</i>	38
Distribution and occurrence of <i>Trichoderma</i>	40
Establishment and proliferation of <i>Trichoderma</i> in soil	41
Biological control approaches with <i>Trichoderma</i>	42
Efficacy of <i>Trichoderma</i> as a biological control agent	46
 <b>III. BIOLOGICAL CHARACTERISTICS OF <i>T. HARZIANUM</i> (RIFAI), <i>T. LONGIBRACHIATUM</i> (RIFAI) AND <i>T. VIRENS</i> (J. MILLER, GIDDENS AND FOSTER) Arx.</b>	
INTRODUCTION	49
METHODOLOGY	
Source of test fungi and pathogen	53
Fungal subcultures	53
Morphological characteristics of <i>Trichoderma</i> colony and cultures	54
Effect of pH: Growth of <i>Trichoderma</i> by mycelial dry weight	56
Effect of pH: Radial growth of <i>Trichoderma</i> by mycelial extension	57
Effect of pH: Spore production of <i>Trichoderma</i> on agar media	58
Enumeration of spore counts by heamocytometer	58
Effect of temperature on growth of <i>Trichoderma</i>	59



Growth inhibition of <i>Ganoderma boninense</i> by dual culture	59
Data analysis	61
RESULTS	
Morphological characteristics of <i>Trichoderma</i> colony and cultures	62
Effect of pH: Growth of <i>Trichoderma</i> by mycelial dry weight	66
Effect of pH: Radial growth of <i>Trichoderma</i> on agar media	71
Effect of pH: Spore production of <i>Trichoderma</i> on agar media	74
Effect of temperature on growth of <i>Trichoderma</i>	75
Screening for antagonistic properties	78
DISCUSSION	82
 <b>IV. THE EFFECT OF INOCULUM CHARACTERISTICS ON THE PATHOGENICITY OF <i>GANODERMA BONINENSE</i> ON OIL PALM SEEDLINGS</b>	
INTRODUCTION	87
METHODOLOGY	
Source of oil palm seedlings, potting media and <i>G. boninense</i> inocula	90
Wood type and its preparation as fungal inocula	90
Method of infecting oil palm seedlings with <i>G. boninense</i>	91
Establishment of disease by two types of inoculum substrate	92
Establishment of disease by smaller sized inoculum substrates	92
Assessment of fungal infection:	93
i. Recognition of Disease Symptoms	93
ii. Scoring by Disease Severity Index (DSI)	94
Data analysis	95
RESULTS	
Disease establishment on 6 x 6 x 12 cm rubber wood inocula	97
Disease establishment on smaller sized inoculum blocks	99
Disease development on seedlings infected by oil palm wood blocks	105
DISCUSSION	108
 <b>V. GREENHOUSE TRIALS: THE APPLICATION OF CONIDIAL SOIL DRENCH OF <i>T. HARZIANUM</i> AS A SINGLE AND AS A MIXED SPECIES INOCULA AND THE SURVIVAL OF CONIDIA IN TREATED SOILS</b>	
INTRODUCTION	111
METHODOLOGY	
Source of fungal cultures	117
Preparation of wood blocks as fungal substrate	117
Preparation of <i>G. boninense</i> substrate inocula	117
Preparation of <i>Trichoderma</i> supplemented mulch	118
Source of oil palm seedlings and potting media	118

Artificial infection of oil palm seedlings	118
Preparation of conidial soil drench	119
Application of <i>Trichoderma</i> conidial soil drench	119
Estimation of <i>Trichoderma</i> spore counts used as conidial soil drench	121
Assessment of fungal infection:	121
i. Recognition of Disease Symptoms	121
ii. Scoring by Disease Severity Index (DSI)	121
Air-dry weight of leaves and roots	122
Determination of soil acidity (pH) and moisture content (MC)	122
Quantification of <i>Trichoderma</i> species from treated pots using colony forming unit (cfu)/g soil	123
Data analysis	125
<b>RESULTS</b>	
Estimation of <i>Trichoderma</i> spore counts used as conidial soil drench	126
Disease establishment on experimental control seedlings	127
Disease establishment on treatment seedlings (T1)	130
Disease establishment on treatment seedlings (T2)	132
Disease establishment on treatment seedlings (T3)	134
Disease severity index	137
Air-dry weight of leaves and roots	139
Determination of soil acidity (pH) and moisture content (MC)	140
Quantification of <i>Trichoderma</i> from soil of treated seedlings by cfu/g soil	142
<b>DISCUSSION</b>	147
<b>VI DISCUSSION AND CONCLUSION</b>	155
<b>REFERENCES</b>	159
<b>APPENDICES</b>	172
<b>BIODATA OF THE AUTHOR</b>	218

## LIST OF TABLES

Tables	Page
3.2 Concentrations of lactic acid and NaOH used to make up the pH readings in agar media	57
3.7 Percentage inhibition of radial growth (PIRG) of <i>G. boninense</i> (EGB 01) and colony degradation time of <i>Trichoderma</i> species against <i>G. boninense</i>	79
4.1 Disease severity index (DSI) based on modifications for oil palm seedlings (Ilias, 2000)	94
4.2 Sequence of signs and symptoms of disease establishment on oil palm seedlings when using 6 x 6 x 12 cm size rubber wood block inocula	98
4.3 Sequence of signs and symptoms of disease establishment on oil palm seedlings when using 6 x 6 x 6 cm size rubber wood block inocula	100
4.4 Sequence of signs and symptoms of disease establishment on oil palm seedlings when using 3 x 3 x 6 cm rubber wood block inocula	103
4.5 Effect of two types of inoculum substrates on oil palm seedlings at 25 weeks after inoculation	105
4.6 Disease severity index (DSI) based on signs and symptoms on oil palm seedlings for a period of 25 weeks	106
5.1 Mean ( $\pm$ standard error) spore counts per ml on heamocytometer of <i>Trichoderma</i> species prior to usage as conidial soil drench for each treatment series for the stated weeks	127
5.2 Sequence of signs and symptoms of disease establishment on oil palm seedlings inoculated with EGB 01 (C2) for a period of 24 weeks	128
*5.3 Sequence of signs and symptoms of disease establishment on oil palm seedlings treated with BIO T32 (T1) for a period of 24 weeks	131
5.4 Sequence of signs and symptoms of disease establishment on oil palm seedlings treated with a mixture of two <i>Trichoderma</i> species (T2) for a period of 24 weeks	133
5.5 Sequence of signs and symptoms of disease establishment on oil palm seedlings treated with a mixture of three <i>Trichoderma</i> species (T3) for 24 weeks	135

5.6	A summary of signs and symptoms of oil palm seedlings with five treatment series at week 24 after inoculation with EGB 01	136
5.7	Disease severity index (DSI) based on signs and symptoms on oil palm seedlings for a period of 24 weeks after inoculation with EGB 01.	138
5.8	Mean ( $\pm$ standard error) air-dry weight of leaves and roots of oil palm seedlings with treatment series at week 24 after inoculation with EGB 01	139
5.9	Mean ( $\pm$ standard error) of soil pH for a period of 22 weeks taken from soils of oil palm seedlings of the five treatment series	140
5.10	Mean ( $\pm$ standard error) of soil moisture content (MC) from control and treated soils of oil palm seedlings taken for a period of 22 weeks	141
5.11	Mean ( $\pm$ standard error) of colony forming units (cfu) of <i>Trichoderma</i> species prior to treatment on the inoculated and non-inoculated oil palm seedlings for a period of 22 weeks at two different depths	142



## LIST OF FIGURES

Figures	Page
3.1 A slide culture chamber.	55
3.2 A seven-day old culture of <i>T. harzianum</i> (isolate BIO T32) on PDA showing the upper surface (L) and lower surface (R).	62
3.3 <i>T. harzianum</i> (isolate BIO T32) culture seen under a microscope at 400X magnification: (a) conidiophore, (b) phialide and (c) a conidium.	63
3.4 A seven-day old culture of <i>T. longibrachiatum</i> (BIO T28) on PDA showing the upper surface (R) and lower surface (L).	64
3.5 <i>T. longibrachiatum</i> (BIO T28) culture seen under a microscope at 400X magnification: (a) conidiophore, (b) phialide and (c) a conidium.	64
3.6 A seven-day old culture of <i>T. virens</i> (BIO T128) on PDA showing the upper surface (L) and lower surface (R).	65
3.7 <i>T. virens</i> (BIO T128) culture seen under a microscope at 400X magnification: (a) conidiophore, (b) phialide and (c) a conidium.	66
3.8 Bars indicate mean mycelial dry weights (g) of the three <i>Trichoderma</i> species cultured in liquid medium at different pH.	67
3.9 Oven-dried BIO T32 mycelia: (a) L – R: pH 2.7, 3.0, 4.0 and 5.0 in three replicates; (b) L – R: pH 5.11 of control; (c) L – R: pH 6.0, 7.0, 7.6 and 8.0 in three replicates.	68
3.10 Oven-dried BIO T128 mycelia: (a) L – R: pH 2.7, 3.0, 4.0 and 5.0 in three replicates; (b) L – R: pH 5.11 of control; (c) L – R: pH 6.0, 7.0, 7.6 and 8.0 in three replicates.	69
3.11 Oven-dried BIO T28 mycelia: (a) L – R: pH 2.7, 3.0, 4.0 and 5.0 in three replicates; (b) L – R: pH 5.11 of control; (c) L – R: pH 6.0, 7.0, 7.6 and 8.0 in three replicates.	70
3.12 Bars indicate mean radial growth (mm/day) of the three <i>Trichoderma</i> species cultured on agar medium at different pH.	72
3.13a Growth response of isolate BIO T32 in a range of pH media at 28°C.	72
3.13b Growth response of isolate BIO T128 in a range of pH media at 28°C.	73

3.13c	Growth response of isolate BIO T28 in a range of pH media at 28°C.	73
3.14	Bars indicate spore production (at spores per ml) of three <i>Trichoderma</i> species cultured in a range of pH 2.7 to 8.0 on PDA.	75
3.15	Bars indicate radial growth rate (mm/day) of three <i>Trichoderma</i> species cultured on agar medium at a temperature range of 15 to 40 °C.	76
3.16a	Growth response of BIO T32 at different temperatures.	77
3.16b	Growth response of BIO T128 at different temperatures.	77
3.16c	Growth response of BIO T28 at different temperatures.	78
3.17a	Inhibition of <i>G. boninense</i> by isolate BIO T32.	80
3.17b	Inhibition of <i>G. boninense</i> by isolate BIO T128.	80
3.17c	Inhibition of <i>G. boninense</i> by isolate BIO T28.	81
4.1	The four disease classes: (a) Class 0 - Healthy palms with green a symptomless leaves; (b) Class 1 - Palms stunted and/or leaf discoloration; (c) Class 2 - Appearance of white mycelia at base of stem; (d) Class 3 - Appearance of sporophore(s) at base of stem and (e) Class 4 – Dead palm with dried up and almost/totally brown leaves, with or without sporophore(s).	96
4.2	Plants infected with 6 x 6 x 12 cm rubber wood block showing a DSI of 85 (bottom row).	99
4.3	Plants infected with 6 x 6 x 6 cm rubber wood block showing a DSI of 40 (bottom row).	101
4.4	Plants infected with 3 x 3 x 6 cm rubber wood block showing a DSI of 35 (bottom row).	104
4.5	Disease progress curves of oil palm seedlings infected with different sizes of rubber wood inocula.	104
4.6	Top row: Seedlings infected with 6 x 6 x 12 cm oil palm wood blocks showing no signs of disease.	106
4.7	Oil palm seedlings infected with oil palm wood blocks inocula (6 x 6 x 6 cm) showing no signs of disease development at 25 weeks of inoculation.	107

5.1	Colony forming units (cfu) of the 3 <i>Trichoderma</i> species on Rose Bengal Agar (RBA) showing different colony characteristics: a) dark green of <i>T. harzianum</i> ; b) pale yellow of <i>T. longibrachiatum</i> and c) greenish-brown of <i>T. virens</i> .	125
5.2	Up-rooted infected, untreated control seedlings (C2) showing a DSI of 86.87 at 24 weeks after inoculation.	129
5.3	Up-rooted uninfected, untreated control seedlings (C1) showing a DSI of 0 at 24 weeks after inoculation.	129
5.4	Up-rooted oil palm seedlings given a single mode (T1) of application with <i>T. harzianum</i> (BIO T32), giving a DSI of 28.34 at 24 weeks after inoculation.	131
5.5	Up-rooted oil palm seedlings given an application of a mixture of <i>T. harzianum</i> and <i>T. longibrachiatum</i> (T2), showing a DSI of 55 at 24 weeks after inoculation.	134
5.6	Up-rooted oil palm seedlings given an application of a mixture of <i>T. harzianum</i> , <i>T. longibrachiatum</i> and <i>T. virens</i> (T3), showing a DSI of 81.66 at 24 weeks after inoculation.	136
5.7	Disease progress curves of oil palm seedlings infected with different sizes of rubber wood inocula.	138
5.8	Colony forming units (cfu/g soil) of BIO T32 from treatment T1 over a period of 22 weeks at two different depths.	144
5.9	Colony forming units (cfu/g soil) of BIO T32 and BIO T28 from treatment T2 over a period of 22 weeks at two different depths.	145
5.10	Colony forming units (cfu/g soil) of BIO T32, BIO T28 and BIO T128 from treatment T3 over a period of 22 weeks at two different depths.	146

## LIST OF ABBREVIATIONS

- G	without <i>Ganoderma boninense</i>
+ G	with <i>Ganoderma boninense</i>
BIO T28	<i>Trichoderma longibrachiatum</i>
BIO T32	<i>Trichoderma harzianum</i>
BIO T128	<i>Trichoderma virens</i>
BSR	Basal stem rot
C1	Control 1
C2	Control 2
$C_6H_8O_7 \cdot H_2O$	Citric Acid
CFU	Colony Forming Unit
DSI	Disease Severity Index
EGB 01	Isolate of <i>Ganoderma boninense</i> ( <i>Elaeis guineensis</i> -Banting)
MC	Moisture Content
MEA	Malt Extract Agar
MPOB	Malaysian Palm Oil Board
MW	Molecular Weight
$Na_2HPO_4$	Sodium Hydrogen Phosphate
NaOH	Sodium Hydroxide
OPMF	Oil Palm Mesocarp Fibre
PCNB	Pentachloronitrobenzene
PDA	Potato Dextrose Agar

PIRG	Percentage Inhibition Radial Growth
PORIM	Palm Oil Research Institute of Malaysia
PP 28	Isolate of <i>Ganoderma boninense</i> from Palm Oil Research Institute of Malaysia (PORIM), now know as Malaysian Palm Oil Board (MPOB)
RBA	Rose Bengal Agar
rpm	Rotation per minute
T1	Treatment 1, <i>T. harzianum</i> (BIO T32)
T2	Treatment 2, <i>T. harzianum</i> + <i>T. longibrachiatum</i> (BIO T28)
T3	Treatment 3, <i>T. harzianum</i> + <i>T. longibrachiatum</i> + <i>T. virens</i> (BIO T128)
UPM	Universiti Putra Malaysia
w.a.i.	Weeks after inoculation
WP	Wettable Powder

# CHAPTER I

## INTRODUCTION

Several million hectares in the world today are planted with commercially important edible oil crops that represent a significant fraction of the resources of the countries concerned (Ariffin and Idris, 2002). Among these species is the oil palm (*Elaeis guineensis*) as an important crop in the topical regions because of its two main raw materials produced, the palm oil and palm kernel oil. Currently, Malaysia is the leading producer of palm oil, with a total production of about 12.5 million tonnes for the year 2004 (MPOB Statistic, 2004) and seeks to maintain dominance in this field.

In order to maintain the current production as well as to strive towards higher yields, every aspect of oil palm cultivation will need to be carefully managed; one of these is in disease management. From seed germination to field planting, the oil palm is prone to attack by various disease-causing organisms, the most common being fungi. Nevertheless, diseases affecting seeds and nursery seedlings are under control in most cases and do not pose a serious threat to the industry. It is diseases of field palms, particularly a basal stem rot (BSR) caused by *Ganoderma* spp. that threaten crop development and requires urgent solution.

*Ganoderma* has been known to attack oil palms since the early years when the crop was introduced into this country (Turner, 1981). The disease was recognized since late 1920's (Thompson, 1931; as cited in Ariffin *et al.*, 1996) but was regarded as of

negligible importance since only palms of over 25 years in age were affected. It was not until 1957 that BSR incidence was reported to increase at an alarming rate when younger palms of 10 to 15 years in age were also infected (Turner and Bull, 1967). Gurmit (1991) reported that the disease could set in as early as 12 to 24 months but the effects were only noticeable when they were four to five years old.

Currently, the approaches used to control the disease are mainly by adoption of hygienic cultural practices and the use of chemical control (tridemorph, carboxin, triadimefon, triadimenol, flutriafol, propiconazole and difenoconazole) to a certain degree (Gurmit, 1991). Bayleton® is one chemical that has been used in laboratory studies and in field trials as trunk injection (PORIM, 1984). Other fungicides tested in field trials were Benlate® T-20, Calixin®, Bayfidan®, Thiram® and Dazomet® but results from these trials were inconclusive (Ariffin and Idris, 1991). Several research institutes have studied this disease and developed means of control but despite many investigations and some 80 years of research no satisfactory solutions in terms of effectiveness, ease of use and cost could be offered.

During the next decade biological control may become an important component of plant disease management practices. The demand for alternatives to chemical control of plant pathogens has become stronger owing to concerns about the safety and environmental impacts of chemicals. The possibility of control of *Ganoderma* should be approached through manipulation of biological agents. Investigations on the use of fungi such as *Trichoderma* (Wijesekera *et al.*, 1996; Ilias and Abdullah,

1999), *Aspergillus* (Shukla and Uniyal, 1989) and *Penicillium* (Dharmaputra *et al.*, 1989) as antagonists of *Ganoderma* in culture have been reported. Particular attention is focused on species of *Trichoderma* that may not as yet given any 'wonder drugs' such as penicillin but has the potential to produce enzymes and to attack or inhibit other fungi (Samuels, 1996; Ilias and Abdullah, 1998; Ilias and Abdullah, 1999). Weindling (1932) was the first to discover the antagonistic ability of *Trichoderma* on the plant pathogen *Rhizoctonia solani*. Two major discoveries were reported; the first was that *Trichoderma* killed the pathogen by physical strangulation and the second was by killing them a short distance away through the production of toxic compounds in the media. Ilias and Abdullah (1998) showed situations where the fungal mycelia coiled tightly around the host hyphae resulting in physical strangulation, as well as the formation of hook-like structures by *Trichoderma*, which puncturing the fungal host cells. *Trichoderma* spp. was also found to produce volatile and non-volatile antibiotics (Dennis and Webster, 1971a,b; Ilias and Abdullah, 1998).

The most studied species of *Trichoderma* acting against antagonists of plant pathogens reported were *Trichoderma harzianum* (Wells *et al.*, 1972; Elad *et al.*, 1980; Chamswarng, 1992; Ilias and Abdullah, 1998), *T. virens* (Papavizas and Lewis, 1989; Sariah and Chan, 1999) and *T. longibrachiatum* (Chamswarng *et al.*, 1992; Sreevinasaprasad and Manibushanrao, 1993; Saravanan *et al.*, 2003). An *in vitro* study by Ilias and Abdullah (1999) showed that growth of *Ganoderma boninense* was inhibited using culture filtrates of *T. harzianum* and *T. virens* respectively. Further *in vitro* studies by Abdullah and Jayanthi (1999) found that a



metabolite mixture of strains of *T. harzianum*, *T. virens* and *T. longibrachiatum* resulted in a better antagonistic performance against growth of *Ganoderma boninense* than when applied singly.

Trials on using disease-controlling agents are still under explored and play an important role in inducing disease control in oil palm seedlings. A major obstacle towards achieving this objective is the inability to reproduce artificial infection accurately and consistently. Studies by Khairudin (1991), was the most successful and practicable thus far and is the model upon which the present study was based. However, the success in establishing induced disease in oil palm seedlings is meaningful only if the data can be quantified. Many attempts have been made in the earlier years to establish Koch's Postulate, one of which was by Navaratnam and Chee (1965). Khairudin *et al.* (1991) found that the oil palm seedlings were infected by rubber wood inocula but not on oil palm mesocarp fibre (OPMF). It was thus concluded that the type of substrate inocula used determined the success of infection by *G. boninense*. Besides the size, type and age of inoculum may also play an important role in establishing infection by *Ganoderma* (Khairudin, 1994; Abdullah *et al.*, 2001).

Based on *in vitro* experiments by Ilias and Abdullah (1998), a further step was taken to test the antagonist activity in greenhouse trials. An *in vivo* trial carried out by Ilias (2000) found a strain of *T. harzianum* used singly in the form of conidial soil drench gave better results in suppressing diseased in oil palm seedlings than *T. virens*.